AD_____

Award Number: DAMD17-01-1-0390

TITLE: Identification of Potential Biomarkers for the Early Diagnosis of Breast Cancer

PRINCIPAL INVESTIGATOR: Peiqing Sun, Ph.D.

CONTRACTING ORGANIZATION: The Scripps Research Institute
La Jolla, California 92037

REPORT DATE: September 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Annual (1 Sep 01 - 31 Aug 02)

4. TITLE AND SUBTITLE
Identification of Potential Biomarkers for the Early Diagnosis of Breast Cancer

5. FUNDING NUMBERS
DAMD17-01-1-0390

6. AUTHOR(S):
Peiqing Sun, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Scripps Research Institute
La Jolla, California 92037

E-Mail: pqsun@scripps.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The successful treatment of breast cancer requires detection of the disease at early stages. Currently the diagnostic tools for breast cancer suffer from drawbacks. In this application, we propose to systematically isolate a panel of secreted and cell surface proteins (trafficked proteins) that are over-expressed in early stage breast tumors, using a novel secretion trap strategy and cDNA micro-array. These proteins are candidates for blood-borne markers and cell surface markers that can be used in the early diagnosis of breast cancer. It is conceivable that multiple blood-borne markers, when used in combination, could predict the existence of tumors more accurately and more successfully. Once the presence of a tumor is predicted, cell surface markers can be used in imaging analysis to identify the location of the lesion. During the first year of the funding period, we have constructed a secretion trap library from multiple breast tumors, and have started the screen to isolated cDNA encoding trafficked proteins.

14. SUBJECT TERMS
mammalian genetics, retrovirus, trafficked proteins, secretion, signal peptide, differential expression, laser capture microdissection, microarray

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

The successful treatment of breast cancer requires detection of the disease at early stages. Currently the diagnostic tools for breast cancer suffer from drawbacks. For example, mammography can miss small lesions, and sometimes can induce tumors in certain patients. Tests of biopsied tumor samples can only be performed in symptomatic patients when the tumor has been identified. Certain blood-borne markers (such as PSA) have been used in affordable, routine serum tests to diagnose cancer in asympotamatic patients. However, these markers, when used individually, can detect the presence of only a certain percentage, but not all, of a particular type of tumors. This argues that a panel of blood-borne markers should be identified and used for the diagnosis of each type of cancer. It is conceivable that multiple blood-borne markers, when used in combination, could predict the existence of tumors more accurately and more successfully. Once the presence of a tumor is predicted, cell surface markers can be used in imaging analysis to identify the location of the lesion.

In this application, we propose to systematically isolate secreted and cell surface proteins (trafficked proteins) with increased expression in early stage breast tumors. These proteins are candidates for blood-borne markers and cell surface markers that can be used in the routine screening of early stage breast cancer. We will first isolate all secreted and cell surface proteins from breast tumors of multiple patients, using a functional approach we have designed and validated. Next, the expression levels of these proteins in normal and early stage breast tumor tissues will be compared, and those with increased expression in tumors will be identified and analyzed. These studies will generate a pool of potential biomarkers, which can be evaluated through further studies for their use in the early diagnosis of breast cancer.

Body

1. Construction of a deep, representational secretion trap library from multiple breast tumors.

In the original proposal, we planed to construct a secretion trap library from 40 breast tumor samples. Due to the availability of tumor samples from the Scripps Clinic, so far we have only been able to collect 20 tumors. A random-primed cDNA library has been constructed in the pTRAPI vector from these tumor samples. Initially, total RNA was isolated from these 20 tumors, and the integrity of RNA was examined by Northern blot analysis using a cDNA fragment encoding GAPDH gene as probe. Five RNA samples were found degraded, and therefore was discarded. Equal amount of each of the remaining 15 samples was combined, and mRNA was subsequently purified by affinity chromatography with oligo-(dT) cellulose resin. Using Nothern blot analysis, we confirmed that the signal representing GAPDH mRNA had been enriched by more than 10 folds in purified mRNA, as compared to the original total RNA sample. cDNA was then synthesized using the Stratagene □–ZAP cDNA synthesis kit. Instead of the oligo-dT primer provided with the kit, we used during cDNA synthesis a random primer containing 6 non-hydrolyzable, thiolated nucleotides. These thiolated nucleotides effectively protected the XhoI restriction site present in the primer from being degraded by DNA polymerase I that was used in the second strand cDNA synthesis. Afte synthesis, cDNA was size-fractionated. cDNA species of 300-2,000 base pairs (bp) in length was selected, and ligated directionally into the EcoRI and XhoI sites of the pTRAPI vector, upstream of the CD8 reporter gene. The ligated library DNA was transformed into DH10B bacterial cells by electroporation, amplified in bacteria and purified in large quantity.

Based on the number of bacterial colonies obtained after the initial transformation of ligated library DNA, we estimated that the primary library contained $1.5X10^7$ independent clones. To confirm the quality of the library, 20 clones was randomly selected from the primary library and evaluated. Restriction analysis using EcoRI and XhoI revealed that 75% (15) of these 20 clones contained cDNA inserts (Fig. 1A). From the sizes of cDNA inserts present in these clones, it was estimated that the average size of cDNA inserts in the library was approximately 430 bp. When digested with EcoRI and XhoI, cDNA inserts released from the total library DNA migrated as a smear on agarose gel, ranging from 100 bp to 2,000 bp in size (Fig. 1B). The lack of discrete bands among the cDNA species suggested that cDNA of different sizes was represented equally in this library. Based on these results, we concluded that the human breast tumor library we had constructed was of good quality and was suitable for the subsequent secretion trap screen.

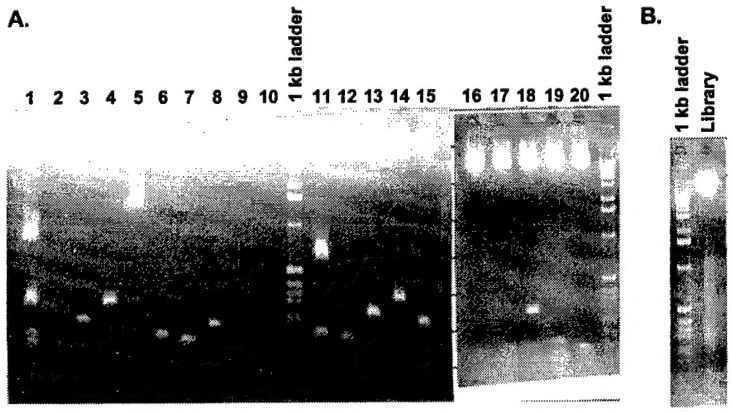

Fig. 1 Restriction analysis of the pTRAP-breast tumor library. A, Plasmid DNA was isolated from 20 randomly selected clones in the library, digested with EcoRI and XhoI to release the cDNA inserts, and analyzed by electrophoresis on 1% agarose gel. Based on the inserts from these 20 clones, it was estimated that 75% (15 out of 20) of the clones within this library contained cDNA inserts with an average size of 430 bp. B., 1 ug of the total pTRAP-breast tumor library DNA was digested with EcoRI and XhoI, and analyzed by electrophoresis on 1% agarose gel.

2). Isolation of cDNA encoding trafficked proteins from the breast tumor library using the secretion trapping screen.

We have started the secretion trap screen. The breast tumor library was transfected into 300 of 10-cm plates of LinXA packaging cells. The viruses were collected and used to infect 300 of 10-cm plates of 293T cells, each containing 10^6 cells. The transduction efficiency was about 20-30%. Therefore, we estimated that 6-9X10^7 of 293 cells was infected with retroviruses, and that this number should be sufficient to cover the breast tumor library that contained 1.5X10^7 clones with at least 4-fold redundancy. The 293 cells are currently under selection with puromycin, in order to purify cells transduced with the library.

Due to the insufficient number of breast cancer patients who underwent surgery at the Scripps Clinic, we have not been able to obtain 40 tumor samples so far for the library construction. Therefore, the current library was derived from 15 patients. We feel that there is a good possibility that these materials are enough to cover the majority of the genetic alterations associated with breast cancer. However, we will continue collecting the tumor samples. Once we acquire 20 more tumors, we will construct another library using the new materials. The new library will be subjected to the CD8 selection as the first library, and the positives obtained from the second screen will be combined with those from the first and arrayed.

Key research accomplishments

1. A deep, representational secretion trap library has been constructed from 15 breast tumor samples.

2. We have started the secretion trap screen to isolate cDNA encoding trafficked proteins.

Reportable outcomes

A random-primed, human breast tumor cDNA library has been constructed in the pTRAPI vector.

Conclusions

We have constructed the secretion trap library from multiple human breast tumors, and have begun the secretion trap screen to isolate cDNA encoding trafficked proteins.

References None.

Appendices None.